DATA ON THE CHARACTERISTICS OF PSEUDOTUBERCULOSIS CULTURES

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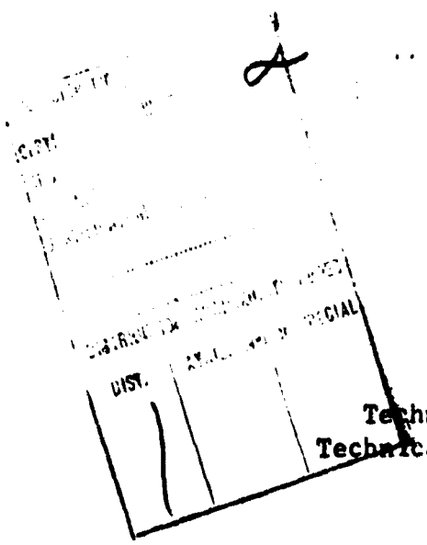

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/Following is the translation of an article by L. A. Timofeyeva and V. Ya. Golovacheva in the Russian-language publication Zhurnal Mikrobiologii, Epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), No 11, 1963, pages 46-51./

From the Irkutsk State Scientific Research Antiplague Institute of Siberia and the Far East

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Isolation of the pseudotuberculosis causative agent from rodents has been described by several authors (Miller and Gladkiy, 1927; Togunova and Migunov, 1930; Tokaravich, et. al, 1940; Dolomanova, 1941; Sergeyeva and Somova, 1956; Somova, Sergeyeva, 1957; Klimova, 1956; Yushchenko, 1957, 1959; Devyadova, Mikhaylova, Yakunina, 1959; Yashchuk, Prokhova, Sovetova, 1960; Klimenko and Klimenko, 1962, etc). No reliable information on pseudotuberculosis in people has been recorded in the Soviet Union. The report of Yurkevich (1911) on the isolation of five pseudotuberculosis cultures from patients ill with inflammation of the lungs by means of test animals occasioned some doubt, since spontanious pseudotuberculosis has been found in guinea pigs. However, the wide distribution of pseudotuberculosis in rodents, and also established cases of pseudotuberculosis with diverse clinical manifestations in persons in France, Sweden, Hungary; Switzerland and other countries (Knapp, 1958; Mollare, 1960) affords grounds to assume the existence among humans of pseudotuberculosis diseases also in

the Soviet Union.

Accordingly, we conducted a comparative study of pseudotuberculosis cultures isolated from various species of rodents and from man.

In the investigation we used 54 pseudotuberculosis cultures isolated from various species of rodents in Siberia and the Far East, and 3 pseudotuberculosis cultures of the serological type I, isolated from persons (kindly sent us by Dr. Mollare from France).

Pseudotuberculosis cultures were isolated from gray and black rats, flat-headed and Eastern voles, house mice trapped out of doors and from guinea pigs and white mice from nurseries (Table 1). The pseudotuberculosis cultures were isolated from trapped wild rodents and various laboratory animals.

TABLE 1

Isolation of Pseudotuberculosis Cultures from Rodents[1]

(a) Вид грызуна	(b) Место выделения	(c) Число штаммов
Серая крыса (d)	(e) Владивосток, Находка, Петропавловск-на-Камчатке, Славянка, Хабаровск, Южный Сахалин	24
Черная крыса (f)	Владивосток (g)	8
Плосковерепная полевка (h)	Монгольская Народная Республика (i)	1
Восточная полевка (j)	Находка (k)	1
Домовая мышь (l)	Советская гавань (m)	1
Белая мышь (n)	Горно-Алтайск, Уссурийск (o)	5
Морская свинка (p)	Владивосток, Горно-Алтайск, Иркутск, Южный Сахалин (q)	14
	(r) Всего . . .	54

[1]Cultures were isolated by physicians of antiplague institutions. Ye. V. Karmanova, O. I. Klimenko, O. A. Mikhaylova, I. G. Fishchenko, A. M. Shamova, T. N. Yakunina, etc.

LEGEND: a) species of rodent; b) location of isolation; c) number of strains; d) grey rat; e) Vladivostok, Nakhodka, Petropavlovsk-na-Kamchatke, Slavyanka, Khabarovsk, Yuzhnyy, Sakhalin; f) black rat; g) Vladivostok; h) flat-headed vole; i) Mongolian People's republic j) Eastern vole; k) Nakhodka; l) domestic mouse; m) Sovetskaya gavan'; n) white mouse; o) Gorno-Altaysk, Ussuriysk; p) guinea pig; q) Vladivostok, Gorno-Altaysk, Irkutsk, Southern Sakhalin; r) Total.

Pseudotuberculosis cultures were isolated from rodents during the entire year.

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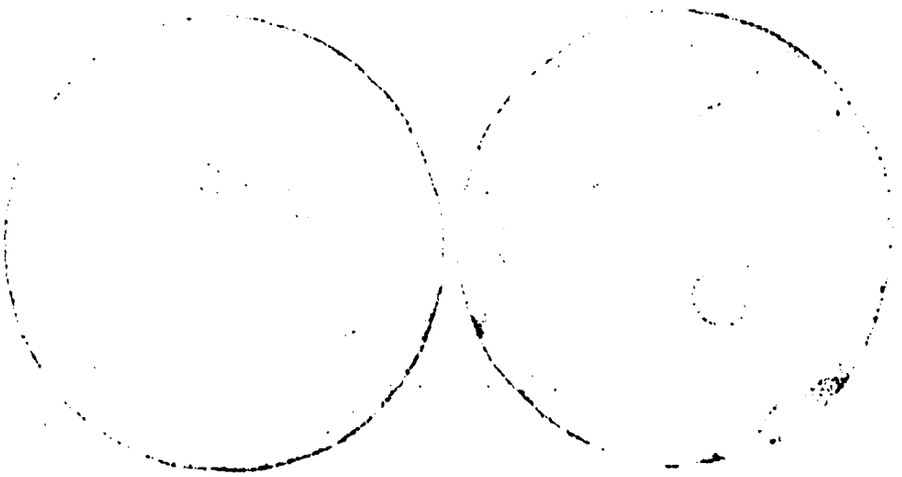

Figure 1. Colonies of pseudotuberculosis bacillus in R- (a) and S- (b) forms. Two-day growth. Magnification: 8X5 and 8X2, respectively.

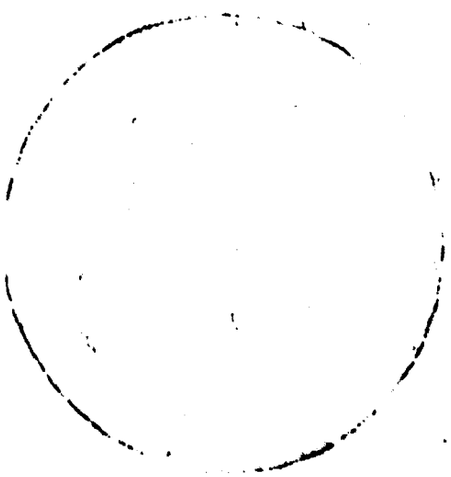

Figure 2. Dissociation of pseudotuberculosis bacillus. Two-day growth. Magnification: 8X5.

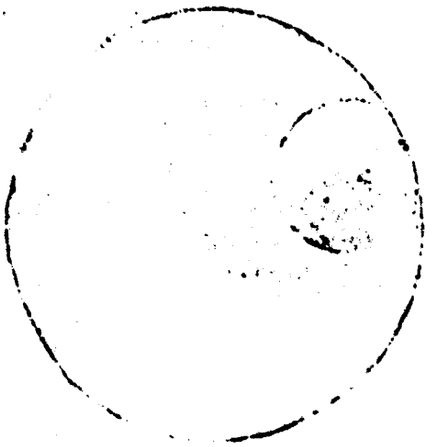

Figure 3. Phagized colonies of pseudotuberculosis bacillus. Two-day growth. Magnification: 8X5.

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The morphology of bacilli isolated from rodents and from man was typical for the pseudotuberculosis causative agent. In preparation stained to Gram they were of the form of Gram-negative short rod-shaped bacilli with rounded ends or coccobacteria.

According to the records of the initial study, bacilli of the freshly isolated cultures on agar plates were of the R- and S- form. In the R-form the colonies were granular or tuberous, with elevated centers, uneven, the periphery more transparent, while in the S-form the colonies were circular, glistening, with light fine-grain centers and with even edges. In the second examination of the resultant cultures following 2-6 months' storage it was established that most cultures were of the R-form (Figure 1a), and some of the S- (Figure 1b), and some of the Or- and Os forms. Upon repeated innoculations in several cultures dissociation (Figure 2) was observed. Most strains contained phage (Figure 3). Our observations indicated that the phagized cultures did not differ in biological properties from the unphagized.

The motility of the cultures was studied in a pendant drop after one day's growing at 22 and 37 degrees on agar plates and in 0.3 per cent semifluid agar (innoculation by injection). Of the cultures grown at room temperature, only 6 strains exhibited active motility, 32 strains were weakly motile, and 19 were nonmotile. Of the cultures raised at 37 degrees, only 10 strains were weakly motile, the remaining 47 strains were nonmotile. Upon frequent investigation of the motility of pseudotuberculosis cultures we noted that this property is not constant. Individual strains under identical conditions of cultivation exhibit more or less pronounced motility or lose it altogether.

In the broth cultivation of 34 strains turbid growth with flocculent precipitate at the bottom of the tube was observed; 23 strains grew with the formation of flocculent precipitate, but the broth itself remained transparent.

The enzymatic activity of the cultures was studied on media containing carbohydrates (D. Andrade indicator and 0.5 per cent carbohydrates). The cultures were grown at 30 degrees. The results were recorded daily for 30 days (Table 2). The biochemical properties of cultures isolated from various species of rodents and man generally agreed: all the cultures studied degraded to acid during first days the sugars glucose, maltose, mannite, ramnose, arabinose, and glactose are fermented during 1-10 days, glycerine -- during 1-5 days, and dextrin was decomposed by 53 strains, whereas 3 strains isolated from the grey rat and 1 -- from the guinea pig, did not ferment dextrin.

Not a single strain fermented lactose, saccharose, dulcite, and sorbite. Alkalinization on litmus milk was noted during 3-10 days, while in Hottinger broth (pH - 7.2, 150 mg% of amine nitrogen) the strains formed hydrogen sulfide during 1-5 days (with the exception

three strains -- two isolated from white mice, and one from guinea pigs) did not coagulate milk, did not form indole and did not dilute gelatine.

All cultures clearly fermented, after 1 day, urea during 1-4 days 2 per cent glycerine was fermented, in 3-24 hours total reduction of methylene blue was attained, and in a day the colored differential medium (Timofeyeva, Aparin, and Golovacheva, 1957) was changed to blue.

All the strains were raised on fasted acidic agar, and upon in nonpeptone agar grew from 15 successive dilutions.

All the cultures exhibited more or less pronounced hemolytic activity with respect to rabbit and guinea pig erythrocytes. Hemolysis was observed in 5 per cent hematic agar at 37 degrees by the third-seventh day. No hemolysis took place in agar to which sheep blood had been added.

All the pseudotuberculosis strains, with the exception of one culture (No 484) isolated from the grey rat were lyzed with pseudotuberculosis phage on solid nutrient media and in broth. The lysis titer of the culture, following Appelman, ranged from 10^{-1} to 10^{-10}.

We must note that two strains not lyzed upon isolation became lyzed after threefold passage in white mice. Four strains not lyzed during the first month following isolation, after storage on semifluid agar for 8-12 months at 18-20 degrees began to clearly be lyzed by pseudotuberculosis phage.

The No 44 culture, in spite of repeated passages through the organism of white mice and nutrient media was not lyzed either by plague or by pseudotuberculosis bacteriophage. In the remaining characters it was typical of pseudotuberculosis bacillus.

Characteristic of most of the isolated cultures was the sensitivity only to pseudotuberculosis bacteriophage, but 13 cultures isolated from grey and black rats in Vladivostok (Devyatova, et. al, 1959) were clearly lived both by pseudotuberculosis as well as plague phage, and they persistently retained this property during 7-10 years.

Baltazard, Davis, Dezignat, et. al. (1956) noted that in raising pseudotuberculosis and plague cultures during 18-20 hours in broth at 18-20 degrees and their subsequent growing on dessicated agar sheets containing plague bacteria phage at 20 degrees for 48 hours, lysis only of plague cultures was observed, while pseudotuberculosis cultures were not. When we used the above method, all 13 strains continued to be lyzed both by plague and by pseudotuberculosis phage.

Study of the ability of pseudotuberculosis cultures to reduce nitrates to nitrites revealed that of 57 strains 55 exhibited a denitrifying capacity, while not one strain was nitrifying.

The ability to nitrify as well as to denitrify was lacking in two cultures isolated from guinea pigs and from grey rats.

Pseudotuberculosis cultures were clearly agglutinated by antiplague serum prepared by the Mikrob Institute: 20 cultures were agglutinated to a titer of (1:2000), 29 -- to half this titer (1:1000), and one strain -- to one-fifth titer (1:400). Only clearly distinct agglutination was counted (+++ and ++++).

Tal' (1954) was the first to serologically classify pseudotuberculosis cultures; according to his data of 186 pseudotuberculosis strains he studied 126 belong to the serological type I, 44 -- to II, 14 -- to III, and only one strain -- to types IV and V.

Girard and Chevalier (1955), studying the serological characteristics of the 56 pseudotuberculosis cultures isolated from various species of animals, birds, and man established that 52 cultures belong to serological type I, two -- to II, and three -- to type III.

For the serological classification of cultures isolated from rodents, we used type sera prepared by the Tal' method and graciously sent us by Dr. Girard from the Pasteur Institute the agglutination reaction was carried out on glass. In a single drop of type serum diluted to 1:10 was suspended a one-day old agar culture grown at room temperature. The reaction was noted in 1-3 minutes (Table 3).

Most cultures (31) belong to serological type I, 8 -- to type IV, one -- to type II, and two -- to type III. Not a single strain was agglutinated by type V serum, and 12 strains were not agglutinated by a single type serum.

The presence of untyped strains gives us grounds to assume that there are still other serological types of pseudotuberculosis cultures which are not agglutinated by the available type sera (according to Tal'), which must serve as a basis for further work.

Pathogenicity for white mice and guinea pigs was studied for most strains isolated from rodents, and for some of the strains we also studied pathogenicity for pigeons. In the study of pathogenicity for white mice and guinea pigs broth cultures were introduced subcutaneously (0.5 ml to white mice and 1 ml to guinea pigs). Two animals were chosen for each dosage. Cultures were administered intramuscularly and intravenously with the pigeons in the amount of 1 billion bacterial cells in a volume of 1 ml.

Most cultures exhibited pathogenicity for white mice. Of 42 strains 33 resulted in the death of white mice in periods ranging from 2 to 13 days; 9 cultures did not induce the death of mice for 30 days. Of 27 cultures 16 proved pathogenetic for guinea pigs; these cultures induced the death of animals in periods ranging from 5-23 days. Of 22 cultures given pigeons intramuscularly, 5 proved to be pathogenic. Pigeons began to die in periods ranging from 3 to 22 days. When cultures were given intravenously to the pigeons in the same doses, of 10 strains 5 induced the death of the pigeons in periods ranging from 2-9 days, and 3 cultures which were non-

TABLE 2

Enzymatic Activity of Pseudotuberculosis Cultures from Various Species of Rodents and Man

(a) Вид животного, от которого выделена культура	(b) Число изученных культур	(c) Ферментация углеводов: (d) глюкоза	(e) лактоза	(f) мальтоза	(g) маннит	(h) сахароза	(i) арабиноза	(j) галактоза	(k) дульцит	(l) декстрин	(m) глицерин	(n) сорбит	(o) рамноза
Серая крыса (p)	24	24/1	—	24/1	24/1	—	21/1; 3/2	22/1; 2/5	—	21/1; 3/0	21/1; 1/2; 2/3	—	24/1
Черная крыса (q)	8	8/1	—	8/1	8/1	—	8/1	8/1	—	8/1	8/1	—	8/1
Плосколобая полевка (r)	1	1/1	—	1/1	1/1	—	1/3	1/10	—	1/1	1/3	—	1/1
Восточная полевка (s)	1	1/1	—	1/1	1/1	—	1/1	1/5	—	1/1	1/3	—	1/1
Домовая мышь (t)	1	1/1	—	1/1	1/1	—	1/2	1/2	—	1/1	1/2	—	1/1
Белая мышь (u)	5	5/1	—	5/1	5/1	—	5/1	4/1; 1/2	—	5/1	3/1; 2/4	—	5/1
Морская свинка (v)	14	14/1	—	14/1	14/1	—	12/1; 1/3; 1/10	14/1	—	13/1; 1/0	8/1; 5/2; 1/3	—	14/1
Человек (w)	3	3/1	—	3/1	3/1	—	3/1	3/1	—	3/1	2/4; 1/5	—	3/1
Всего. (x)	57	57/1	—	57/1	57/1	—	50/1; 4/2; 2/3; 1/10	51/1; 2/2; 3/5; 1/10	—	53/1; 4/0	40/1; 7/2; 5/3; 4/4; 1/5	—	57/1

LEGEND: a) species of animals from which culture was isolated; b) number of cultures studied; c) fermentation of carbohydrates; d) glucose; e) lactose; f) maltose; g) mannite; h) saccharose; i) arabinose; j) galactose; l) dextrin; m) glycerin; n) sorbite; o) ramnose; p) gray rat; q) black rat; r) flat headed vole; s) Eastern vole; t) house mouse; u) white mouse; v) guinea pig; w) man; x) total

TABLE 3

Serological Classification of Pseudotuberculosis Cultures Isolated from Various Species of Rodents

a) Вид грызуна, от которого выделена культура	b) Число исследованных культур	c) Серологические типы по Талю					
		I	II	III	IV	V	d) нетипирующиеся
e) Серая крыса	24	15	1	—	4	—	4
f) Черная крыса	8	—	—	—	4	—	4
g) Плоскочерепная полевка	1	1	—	—	—	—	—
h) Восточная полевка	1	1	—	—	—	—	—
i) Домовая мышь	1	1	—	—	—	—	—
j) Белая мышь	5	4	—	—	—	—	1
k) Морская свинка	14	9	—	2	—	—	3
l) Итого.	54	31	1	2	8	—	12

LEGEND: a) species of rodent from which culture was isolated; b) number of cultures investigated; c) serological types according to Tal'; d) untyped; e) grey rat; f) black rat; g) flat-headed vole; h) Eastern vole; i) house mouse; j) white mouse; k) guinea pig; l) total

pathogenic for intramuscular administration were pathogenic for intravenous.

Upon dissection, enlarged liver and spleen was noted in the dead mice, and some swelling of the regional and inguinal glands.

Suppurative-necrotic inflammatory foci were detected in perished guinea pigs at the site of culture administration, regional and other lymph nodes were moderate in size, and sometimes quite enlarged, the liver appeared swollen and plethoric, in addition, numerous fine necrotic foci were detected under the capsule and in microscopic cross section, the spleen appeared swollen and contained small whitish foci in cross section, the lungs appeared to be hyperemic, the adrenals were mildly enlarged in volume, and sometimes some hyperplasia of lymph formations of the ileum was observed.

In guinea pigs sacrificed with chloroform on the 30th day a slight enlargement of the lymph nodes could be noted, some of which were quite large (containing a hard grain [prosyanoye zerno]) whitish nodules in the liver, and a slight swelling of the spleen.

A culture of pseudotuberculosis bacillus was isolated from a suspension of the organs of all perished and most of the chloroform-sacrificed animals.

The change in the pathologo-anatomical picture in chloroformized animals and isolation of culture therefrom evidences that the rodents can be bacterio-carriers of pseudotuberculosis infection.

CONCLUSIONS

1. In studying pseudotuberculosis cultures isolated from various species of rodents in the Far East and in Siberia, and cultures isolated from man (sent from France), the identity of their cultural-biochemical properties was established.

2. In serological study of 54 strains isolated from rodents, using typical pseudotuberculosis sera 31 were grouped in serological type I, 1 -- in type II, 2 -- in type III, 8 -- in type IV, and 12 were not agglutinated by type sera.

3. The extensive distribution of pseudotuberculosis infection among rodents and the identity of the biological properties of strains isolated from rodents and from man allows us to regard rodents as one of the reservoirs of pseudotuberculosis infection in man.

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